LOSS OF THE PROTEIN IFT57 REDUCES INTRAFLAGELLAR TRANSPORT COMPLEX B STABILITY IN *CHLAMYDOMONAS REINHARDTII*

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Loss of the Protein IFT57 Reduces Intraflagellar Transport Complex B stability in *Chlamydomonas reinhardtii*

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The intraflagellar transport (IFT) protein complex is a vital component needed for the maintenance and generation of the cellular organelle flagellum or cilium. This process is termed ciliogenesis. The complex is comprised of two sub-complexes, A and B, each containing multiple protein subunits that are responsible for the delivery of flagellar assembly proteins along the proximal and distal end of the flagella organelle. *Chlamydomonas reinhardtii* is used as a model organism for studying the IFT machinery as it possesses a highly conserved IFT protein complex similar to that in humans. Previous research has shown that defects in the flagellar assembly process and in the IFT machinery, may lead to human diseases termed ciliopathies. The aim of this study is to utilize an IFT complex B mutant (*2p40*) deficient in the protein IFT57 to determine this protein's role in the proper function of the IFT complex and ciliogenesis. Data characterizing this IFT57 mutant shows complex B proteins are highly reduced in *2p40* when compared to wild type cells. A western blot assay of the mutant was used to determine if IFT57 does or does not affect the stability of the IFT complex. The data suggests the reduced level of IFT57 within the cell reduces the stability of the IFT B complex. Previous and ongoing research shows IFT57 is involved in the pathology of the human ciliopathy Huntington's disease; therefore, further investigation of IFT57's role in the IFT complex and ciliogenesis could aid in the innovation of ways in which to treat this disease.

DEDICATION

I would like to dedicate this thesis to my parents and siblings.

ACKNOWLEDGMENTS

I would like to thank Dr. Hongmin Qin for allowing me to do undergraduate research in her lab. I would also like to thank Xue Jiang for her help and collaboration on this project. Thanks to my sister who I started this project with.

NOMENCLATURE

IFT	Intraflagellar transport
Wt	*Chlamydomonas reinhardtii* mating type + Wild type cells
Hygr	Hygromycin B antibiotic
RESDA	Restriction Enzyme Site Directed Amplification
PCR	Polymerase Chain Reaction
TAP	Tris-acetate-phosphate media
M1	Minimal media 1
bp	Base pair

CHAPTER I

INTRODUCTION

The cilia, or flagella, organelle is a vital cell structure as it provides sensory and motor function for the cell (Lee et al., 2015). These functions are necessary for the day to day life of most cells as is the case for the unicellular green algae, *Chalmydomonas reinhardtii*, which depends on this ability to properly perform phototaxis. The function of the IFT protein complex is to provide the generation and maintenance of the cilia organelle (Qin et al., 2003). By default, organisms with flagella like *C. reinhardtii* are dependent on the IFT complex to function properly. When this system breaks down the consequences have negative downstream systematic effects which are classified as ciliopathies in organisms such as humans (Ishikawa and Marshall, 2011). These ciliopathies include: polycystic kidney disease, retinal degeneration, and Bardet-Biedl syndrome (Badano et al., 2006). These diseases emphasize the need to further study the IFT complex. In this study I isolated a flagellar defective mutant. Our lab utilizes insertional mutants that have linearized pHyg3 plasmid inserted somewhere within the *Chlamydomonas reinhardtii* genome. Insertional mutant *2p40* (*ift57*) was selected at random for testing using restriction enzyme site-directed amplification (RESDA) polymerase chain reaction (PCR). Using this method, I mapped the mutated gene encoding the IFT particle complex B protein, IFT57. The pHyg3 insertion and the IFT57 gene are depicted in Figure 1. I confirmed the mutant failed to express IFT57 by western blotting. Phenotypically, *ift57* mutant *2p40* is mostly bald and in clusters when cultured in liquid TAP growth media. In M1 growth media bubbling systems the mutant is able to form near full length flagella. The unique phenotype of this flagellar mutant can provide further insight into the role of IFT57 in the cell maintenance of the flagellum. Furthermore, IFT57 is

implicated in the pathology of Huntington's disease (Karam et al., 2015). Continued study of mutant *ift57* could help to better understand the pathology of this disease.

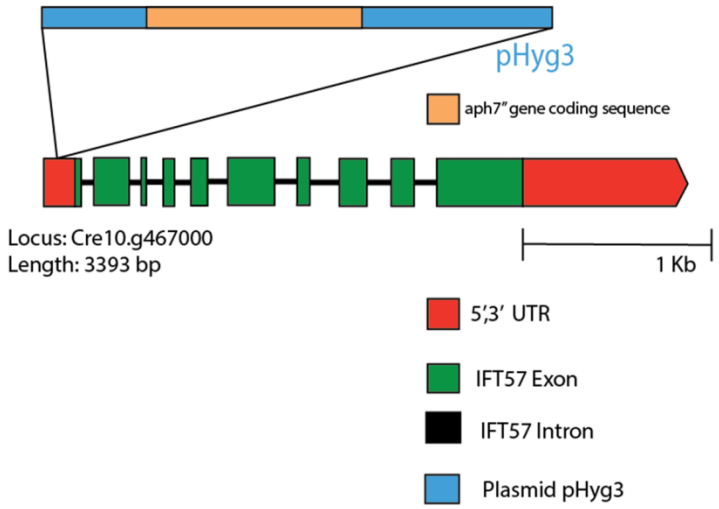

Figure 1**.** *ift57* (2p40) with insertional mutation pHyg3**.** The figure of the IFT57 gene (length 3393 bp) shows the gene with the insertional mutation generated by our lab. The insertional plasmid pHyg3 was linearized and inserted into the promoter region of IFT57. The exons, introns, and untranslated regions (UTR) are depicted. Also depicted is the linearized pHyg3 plasmid which contains the coding sequence for hygromycin resistance.

CHAPTER II

MATERIALS AND METHODS

PCR

PCR was used to demonstrate that *ift57* has the insertional fragment pHyg3 which disrupts IFT57 levels within the cell. Primers Left 1 and Right 1 were synthesized to amplify the IFT57 gene region. I performed PCR using DNA taken from *cc125* and *ift57*.

Western blot assay

Results from the sequencing of our PCR products demonstrated that our mutant *ift57* was an IFT57 mutant. In order to confirm these results we ran a western blot assay on protein samples from cc125 and *ift57* using antibodies specific to IFT57 and other complex B proteins.

Western blot of cycloheximide treated cells

The question of if the stability of the IFT complex is affected by the absence of IFT57 will be determined using a western blot assay of Cycloheximide treated cells. The experiment consists of treating the IFT57 mutant *ift57*, and positive and negative control strains with the protein biosynthesis inhibitor, Cycloheximide. This treatment will be done at three time intervals of zero, six, and twelve hours. The purpose of the time intervals is to show a trend of protein degradation over time; in other words, if the complex is destabilized then there will be a trend of decreasing protein levels. This trend will be visualized using a western blot assay of whole cell level protein extractions taken from each sample at the respective time points. The samples to be tested are mutant *ift57*, and wild type (*cc125*) as the positive control.

CHAPTER III

RESULTS

The results from the RESDA-PCR sequencing demonstrated that the mutant I had selected was an IFT57 mutant. To confirm these results we performed a western blot assay on whole cell protein extracts tagged with an IFT57 antibody as shown in Figure 2. In addition to testing the levels of this protein, I tested the levels of other IFT complex proteins and mutants to compare our mutant with other mutants from our lab.

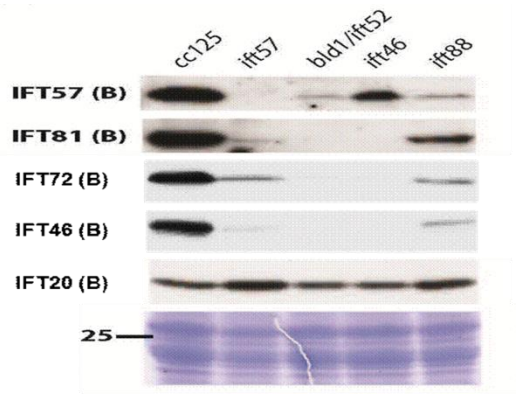

Figure 2**.** Western blot assay**. A** western blot assay of multiple IFT complex B proteins including IFT57 is depicted. The test for each protein was performed using classical western blot techniques and using the antibody necessary for each protein. The assay was testing the relative amounts of each protein present at the whole cell level for several mutants and *cc125* as a control.

Whole cell levels of IFT57 and other IFT complex B proteins in our mutant were highly reduced when compared to wt cells. In addition to our western blot assay, I performed a PCR with

primers synthesized to amplify the IFT57 gene, Left 1 and Right 1. This was done to determine whether or not the mutant possessed the insertional mutation. The results from the PCR can be seen in Figure 3.

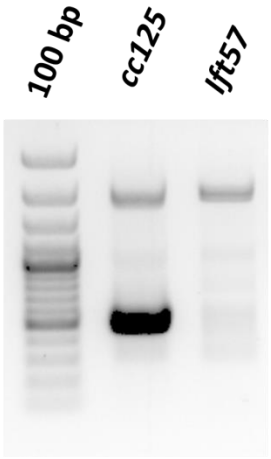

Figure 3. PCR with left 1 and right 1 primers. IFT57 gene specific primers were used in this PCR program, Left 1 and Right 1. Gel was run on 1% agarose gel at around 95 Volts. 100 bp molecular weight standard loaded in the far left lane. Primers were used to demonstrate the presence of the insertional mutation in our mutant *ift57*.

Absence of the band for *ift57* demonstrates the presence of the insertional mutation. The results from these two tests suggest IFT57 may play a role in the stability of the IFT B complex. To test whether or not IFT57 does play a role in stabilizing the IFT B complex I performed a western blot assay on cycloheximide treated cells. The cycloheximide acts as a protein biosynthesis inhibitor (Ma et al., 2013). We tagged the protein extracts with an IFT46 protein antibody which is a complex B core protein (Hou et al., 2007). This was done to show the destabilization of the complex B core. The results from this assay can be seen in Figure 4.

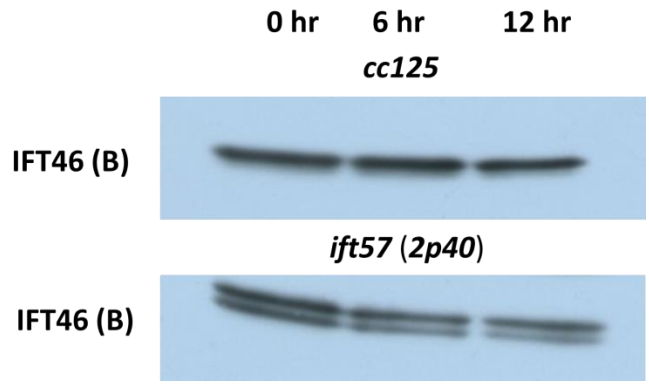

Figure 4. Western blot assay of cycloheximide treated cells. A western blot assay using IFT46 complex B protein antibody is depicted. Protein samples were taken from cells treated with cycloheximide to inhibit protein synthesis. Samples were taken at different intervals during treatment.

The results from the cycloheximide experiment show a decrease in the levels of the IFT B complex core protein, IFT46, for the mutant *ift57* after the twelve hour period. The results show no change in protein levels for the wt strain *cc125*.

CHAPTER IV

DISCUSSION

The data compiled leads to a greater understanding of our mutant, *ift57* (*2p40*). Based on the results of our western blot assays, IFT57 is crucial to the stability of the IFT B complex. This is demonstrated by the decreased levels of IFT B complex proteins in our mutant and is confirmed by the change in levels of IFT46 in the cycloheximide experiment. The reasons behind IFT57's absence causing such a dramatic decrease in other IFT B complex protein levels is the subject of further study. Mainly we would like to consider how exactly IFT57's absence has affected the other B complex proteins at the transcriptional and the translational level. Another direction for this project is to test the extent of ift57's ciliary defects; for this reason there is precedent to test the level of IFT protein inside the flagella which could help us find the location in the cell where IFT57 affects the IFT complex assembly. With this we aim to further our understanding of the IFT complex and flagellar stability. Furthermore, IFT57's role in the pathology of Huntington's disease emphasizes the need to further study the effects of this protein.

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